THE EFFECT OF BEETROOT JUICE ON INTERMITTENT SHUTTLE RUNNING PERFORMANCE INVOLVING DIFFERENT NUMBERS OF DIRECTIONAL CHANGES

by

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Abstract

The aim of the study was to assess the effect of dietary nitrate (NO_3^-) supplementation on blood pressure and the physiological responses to submaximal shuttle running and performance during intermittent shuttle running involving different numbers of directional changes. Eight male recreational team sport athletes (age: 22.6 ± y, body mass: 79.4 ± 4.4 kg, stature: 179.4 ± 5.4 cm, predicted VO_{2max}: 48.5 ± 4,1 ml·kg·$^{-1}$·min·$^{-1}$) completed submaximal shuttle running at 60% of their pre-determined VO_{2peak} and intermittent shuttle running to exhaustion over a 20 m course or a 10 m course involving more directional changes. Participants performed each protocol twice across four trials; once following the ingestion of NO_3^- concentrated beetroot juice 2.5 h before exercise and once following the ingestion of NO_3^- depleted beetroot juice. Oxygen uptake (VO_2), heart rate (HR), rating of perceived exertion (RPE), blood lactate and time to exhaustion during intermittent shuttle running were assessed. Increasing the number of directional changes increased the VO_2 and HR response to submaximal shuttle running ($p < 0.05$). However, NO_3^- did not affect blood pressure, the physiological responses to submaximal exercise or performance during intermittent shuttle running ($p > 0.05$). These findings indicate that increasing the number of directional changes during shuttle running elevates the physiological and metabolic demand, but that NO_3^- does not impact upon the physiological responses or performance during submaximal and intermittent shuttle running.

No portion of the work referred to in this Research Project has been submitted in support of an application for another degree or qualification of this, or any other University or institute of learning.

The project was supervised by a member of academic staff, but is essentially the work of the author.

Signed …….

Date ……

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Chapter 1.0

Introduction

Dietary nitrates (NO_3^-) are found in high quantities in green leafy vegetables and organic beetroot juice (Jones, 2014; Lidder & Webb, 2013). When ingested, the majority of NO_3^- is absorbed in the gastrointestinal tract, whilst some (25%) is reduced to nitrite (NO_2^-) in the oral cavity, with plasma NO_3^- and NO_2^- levels peaking at 1-2 hours and 2-3 hours after ingestion, respectively (Lundberg & Weitzberg, 2009). Once in blood plasma, NO_2^- serves as a precursor and substrate for the endogenous production of nitric oxide (NO) via a one electron reduction process catalysed by numerous reductase enzymes (Jones, 2014; Lundberg, Weitzberg & Glaswin, 2008). This pathway of NO production is facilitated under conditions of low muscle pH and O_2 tension (Lundberg et al., 2008; Bailey et al., 2012), and serves as an alternative pathway to the oxygen dependent synthesis of NO via the oxidation of L-arginine by nitric oxide synthase enzymes when this pathway might become limited during conditions of low oxygen availability, such as those encountered during intense exercise (Lundberg et al., 2008; Moncada & Higgs, 1993). NO plays numerous roles in promoting optimal physiological functioning, such as improving vasodilation, increasing mitochondrial efficiency and calcium handling and delaying human muscle fatigue (Jones, 2014; Bailey et al., 2010; Hernandez et al., 2012).

Numerous studies have demonstrated that supplementing with dietary NO_3^- improves exercise performance in humans (Van de Walle & Vukovich, 2018; Dominguez et al., 2018). Larsen, Weitzberg, Lundberg & Ekblom (2007) were the first to investigate the effects of NO_3^- on exercise performance. In their original study, it was demonstrated that three days of supplementation with NO_3^-, in the form of 0.1 mmol of sodium nitrate, reduced the oxygen cost of cycling by 5% at various

submaximal workloads. These findings demonstrated that NO_3^- could improve one of the key determinants of exercise performance - exercise efficiency - and initiated much research interest into the role of NO_3^- as an ergogenic aid. Several studies have corroborated these original findings of a reduced O_2 cost of exercise and demonstrated that NO_3^- supplementation improves exercise tolerance during fixed intensity exercise (Bailey et al., 2009; Breese et al., 2013; Vanhatolo et al., 2010) and improves exercise performance during self-paced time-trials typical of race performance (Lansley et al., 2011; Cermak, Gibala & Van Loon, 2012; Macleod et al., 2015). In contrast, some studies have reported that the O_2 cost of exercise and performance remain unchanged following NO3- ingestion (Cermak et al., 2012; Wilkerson et al., 2012; Peacock et al., 2012). This equivocacy may be explained by the differences in training status between participants and the different dosing regimens used by studies (Jones, 2014; Porcelli et al., 2015). Indeed, well-trained participants appear to display a lower responsiveness to NO_3^- supplementation, due to higher initial plasma NO_2^- levels. Furthermore, greater muscle oxygenation levels in these athletes may inhibit the reduction, of NO_2^- to NO (Dominguez et al., 2018; Porcelli et al., 2015; Poveda et al., 1997), and a higher NO_3^- dose may be needed in well-trained individuals. The intensity of the exercise protocol used in studies also appears to play an important role in determining the ergogenic potential of NO_3^-. Indeed, NO_2^- appears to improve exercise performance during exercise performed at greater intensities, which likely reflects the higher order motor unit recruitments and the associated greater reduction in muscle pH and O_2 tension, which enhance the reduction of NO_2^- (Jones, 2014; Lundberg et al., 2008; Shannon et al., 2017).

During team sports, athletes perform intermittent exercise characterised by periods of high intensity exercise efforts interspersed with low intensity efforts

(Chandler, Pinder, Curran & Gabbett, 2014; Bradley et al., 2009). The transition from low to high intensity efforts solicits a high contribution from type II motor units and results in significant hypoxia and acidosis (Thompson et al., 2015; Bangsbo, 2000), providing conditions for facilitated NO_2^- reduction to NO. Numerous studies have demonstrated that NO_3^- supplementation improves exercise performance during intermittent exercise protocols designed to replicate the demands of team sports (Dominguez et al., 2018; Wylie et al., 2016; Wylie et al., 2013; Thompson et al., 2015). For example, Wylie et al. (2013) demonstrated that the daily ingestion of 16.2 mmol of NO_3^- in the form of beetroot juice for three days preceding a YO-YO IR1 test increased distance covered by 4.2%, indicating that NO_3^- supplementation may improve team sports performance.

In-between repeated intermittent efforts, team sports athletes perform numerous directional changes and accelerations and decelerations (Bloomfield, Polman & Donoghue, 2007). Unlike during constant speed running, accelerated and decelerated running phases preclude the attainment of a steady state VO_2 response and such movements impose a higher metabolic and energetic demand that is proportional to the rate and demand of the acceleration (Osgnach, Poser, Bernardini, Rinaldo & Di Prampero, 2010; Di Prampero et al., 2012). Indeed, increasing the number of directional changes and accelerations in constant speed and intermittent shuttle running protocols has been demonstrated to lead to a greater VO_2, blood lactate and RPE response (Buchheit, Haydar, Hader, Ufland, Ahmaidi, 2011; Ashton & Twist, 2015; Dellal et al., 2010), indicating a higher metabolic and perceptual demand, which may result from a higher recruitment of type II motor units associated with breaking and re-acceleration (Ashton & Twist, 2015; Dellal et al., 2010). The greater muscle acidity resulting from the higher type II fibre recruitment may enhance

the endogenous reduction of NO_2^- to NO. Furthermore, a strong body of evidence suggests that NO_3^- may improve type II muscle fibre functioning by preferentially increasing blood flow and improving calcium handling in these fibres (Hernandez et al., 2012; Ferguson et al., 2012; Jones, Ferguson, Bailey, Vanhatolo & Poole, 2016). Thus, it is plausible that NO_3^- supplementation may be more ergogenic during exercise involving greater number of directional changes and accelerated running phases.

Accordingly, the aim of the current study was to investigate the effects of NO_3^- supplementation on the physiological responses to constant speed shuttle running and on performance during an intermittent shuttle run test, with both protocols manipulated to involve different numbers of directional changes and acceleration phases.

Chapter 2.0

Methods

Participants

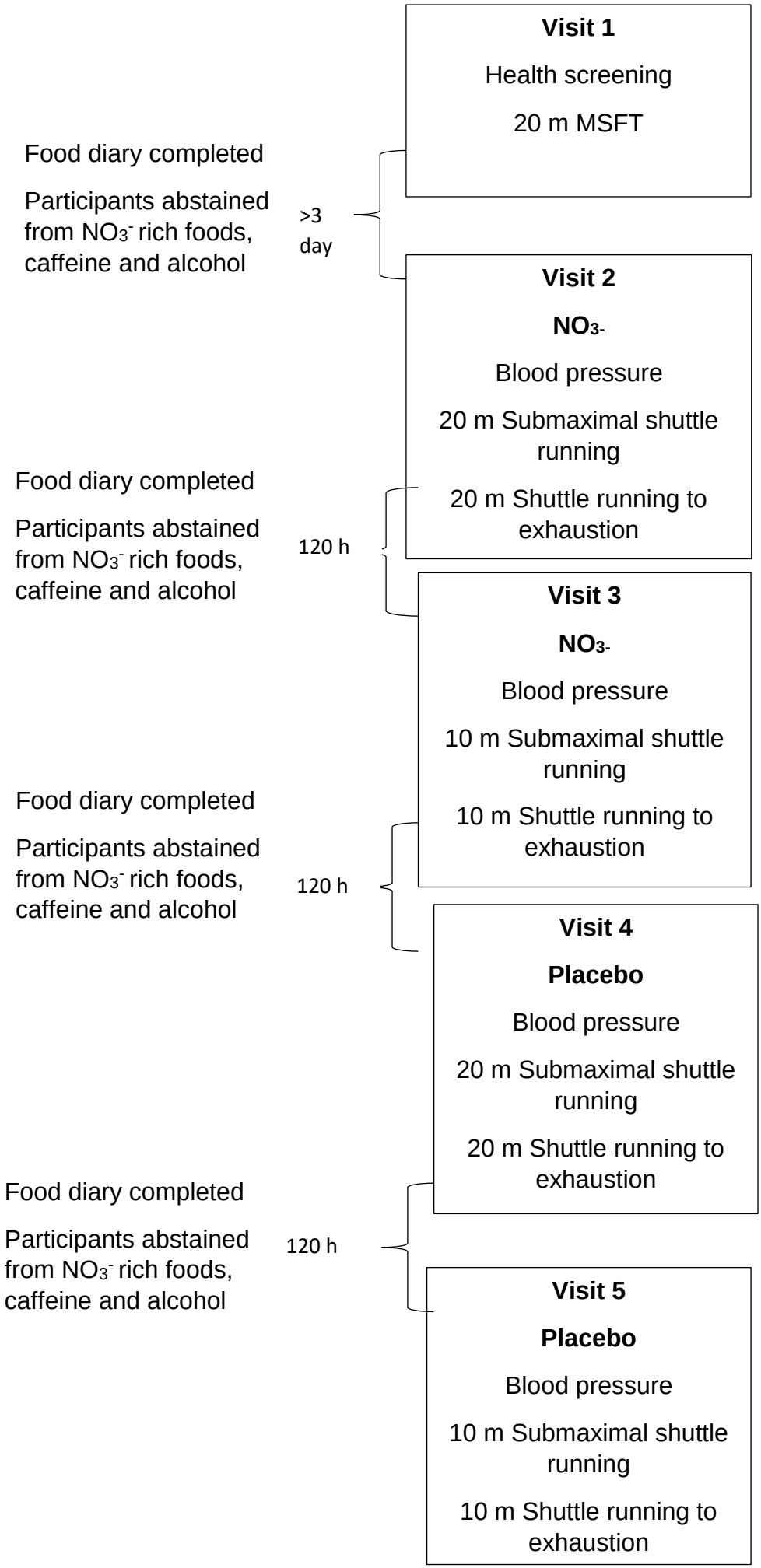
Before data collection, ethical approval for the study was sought from the ethics committee for the Faculty of Dentistry, Medicine and Life Sciences at The University of Chester (see appendix 1). Upon ethical approval, eight moderately trained male team sport athletes who predominantly participated in football at a recreational level (age: 22.6 ± y, body mass: 79.4 ± 4.4 kg, stature: 179.4 ± 5.4 cm, predicted VO_{2max}: 48.5 ± 4.1 ml·kg·$^{-1}$·min·$^{-1}$) were recruited to participate in the study. All participants completed a health screening questionnaire and provided written informed consent before beginning the investigation (appendix 3).

Study design

A schematic of the study design is presented below in figure 1. The study employed a double-blind, repeated measures, randomised crossover design. On the first visit, participants completed a 20 m multi-stage fitness test (MSFT) to determine aerobic capacity. During this visit, participants also performed a familiarisation trial of the 10 m version of the intermittent shuttle run test to exhaustion. Participants then completed the following four experimental trials on separate days in a random order: 1) 20 m submaximal and intermittent shuttle running to exhaustion with prior nitrate supplementation; 2) 20 m submaximal and intermittent shuttle running with placebo; 3) 10 m Submaximal and intermittent shuttle running with nitrate and; 4) 10 m submaximal and intermittent shuttle running with placebo. Each experimental trial was separated by five days to allow sufficient time for plasma NO_3^- and NO_2^- levels to return to baseline values (Jones, 2014). Resting blood pressure was assessed approximately 5 minutes prior to beginning exercise during each trial as an indirect

marker of the increase in plasma NO_{2}^{-} levels and vasodilation following NO_{3}^{-} ingestion (Larsen et al., 2006).

Participants were asked to arrive for testing in a fully hydrated and rested state and were asked to refrain from any strenuous exercise in the 48 h period preceding exercise. Participants completed a food diary in the 24 hours leading up to the exercise trials and were asked to consume a similar diet in-between experimental visits Furthermore, participants were provided with a list of NO_{3}^{-}-rich foods (see appendix 4), which they were asked to abstain from throughout the duration of the study. Participants were also asked to refrain from caffeine, alcohol and anti-bacterial mouthwash (Govoni, Jansson, Weitzberg, & Lundberg, 2008) during the investigation.


Visit 1
Health screening
20 m MSFT
Food diary completed
Participants abstained from NO_3^- rich foods, caffeine and alcohol
>3 day
Visit 2
NO_3^-
Blood pressure
20 m Submaximal shuttle running
20 m Shuttle running to exhaustion
Food diary completed
Participants abstained from NO_3^- rich foods, caffeine and alcohol
120 h
Visit 3
NO_3^-
Blood pressure
10 m Submaximal shuttle running
10 m Shuttle running to exhaustion
Food diary completed
Participants abstained from NO_3^- rich foods, caffeine and alcohol
120 h
Visit 4
Placebo
Blood pressure
20 m Submaximal shuttle running
20 m Shuttle running to exhaustion
Food diary completed
Participants abstained from NO_3^- rich foods, caffeine and alcohol
120 h
Visit 5
Placebo
Blood pressure
10 m Submaximal shuttle running
10 m Shuttle running to exhaustion

Multi-Stage Fitness Test

To predict VO_{2peak} and the speed corresponding to this value, participants completed the MSFT according to the procedures described by Leger, Mercier, Gadoury & Lambert (1988). Participants ran along a linear 20 m course a speed dictated by an audio signal. The test commenced at a speed of 8.5 km/h and increased by 0.5 km/h at each minute. Participants were instructed to run to exhaustion and were provided with strong verbal encouragement throughout the test. The test was terminated when the participant became volitionally exhausted or was unable to maintain the required running speed and failed to meet the beep over two consecutive shuttles. The level achieved at exhaustion was used to predict VO_{2peak} based by matching the level and the number of shuttles completed at exhaustion to the corresponding VO_{2max} value provided in the table provided by Leger et al. (1988). The purpose of this was to calculate 60% of the speed at participants' VO_{2peak}, which was used for the submaximal shuttle run trials. This testing protocol has previously been shown to be a valid and reliable method of estimating maximal oxygen uptake values in a healthy adult population (Ramsbottom, Brewer & Williams, 1988; Leger & Gadoury, 1989).

Submaximal and intermittent shuttle running

An illustration of each testing visit is provided below in figure 2. All shuttle running trials were performed in an indoor sports hall. Before shuttle run tests, participants were permitted to perform any warm-up activities or stretching they desired and were asked to keep these consistent across trials. To investigate the effects of NO_3^- on the physiological and perceptual responses to submaximal shuttle running, participants performed constant speed shuttle running between two cones

along a linear course at 60% of the speed corresponding the speed at VO_{2peak}. This exercise intensity was chosen based on previous studiers which used a similar exercise intensity to investigate the effects of NO_3^- ingestion on oxygen uptake during submaximal exercise (Bailey et al., 2009; Vanhatalo et al., 2010) and in order to be similar to the average intensity recorded during team sports (Bangsbo, Mohr & Krustrup, 2006). Participants completed a 20 m version of the protocols and a 10 m version, where the cones were set 10 m apart and participants were required to decelerate at the 10 m point, before changing direction and reaccelerating to achieve the required running speed. This modification has been used in a previous study to manipulate the number of directional changes during the Loughborough intermittent shuttle run test (Ashton & Twist, 2015). Oxygen uptake (VO_2) and heart rate (HR) were measured continuously throughout the protocol using a portable gas analysis system (K5, Cosmed, Rome), with values averaged values over the final minute used for analysis. In a pilot study, oxygen uptake values produced during this protocol demonstrated good inter-trial reliability when the trials were separated by two days (CV: 4.7%). Participants were asked to provide a rating of perceived exertion (RPE) in the final minute of each run using Borg's RPE scale (Borg, 1988), and a capillary blood sample was taken from participants' fingertip three minutes after exercise and assessed for blood lactate concentration (Lactate pro, Kyoto, Japan).

After a 10 minute recovery period from the submaximal shuttle running protocol, participants completed part B of the Loughborough intermittent shuttle run test (Nicholas, Nuttall & Williams, 2000). Run times to exhaustion during this test have previously been reported to be highly reproducible, showing narrow 95% limits of agreement (-3.19 to 2.16) (Nicholas et al., 2000). Participants were instructed to run between two cones, with the speed alternating between 55% and 95% of the speed

corresponding to their predicted VO_{2peak}, as determined by the 20 m MSFT on visit 1. Participants were instructed to run until they become exhausted and the test was terminated when participants were no longer able to maintain the speed dictated by the audio tape. Participants were provided with strong verbal encouragement throughout the test, which was kept consistent across trials (Midgley, Marchant & Levy, 2018). VO_2 and heart rate were measured continuously throughout the protocol and participants provided an RPE at each minute. All physiological and perceptual variables were averaged out across the protocol for analysis. A capillary blood sample was collected three minutes upon completion of the protocol and was analysed for blood lactate concentration.

Supplementation

In a double-blind fashion, two hours before testing, participants ingested a 70 ml NO_3^- concentrated beetroot shot (containing 6.4 mmol of NO_3^-) (James White drinks, UK) or a NO_3^- depleted beverage (containing 0 mmol of NO_3^-). Both supplements were contained in the same packaging and were taste-matched. The timing and dose of NO_3^- ingestion was based on previous studies showing that an acute moderate dose of NO_3^- improves exercise performance (Van de Walle, 2018; Vanhatolo et al.,2010), and so that the performance of the exercise trials would coincide with the peak in plasma NO_2^- levels (Lundberg et al., 2008).

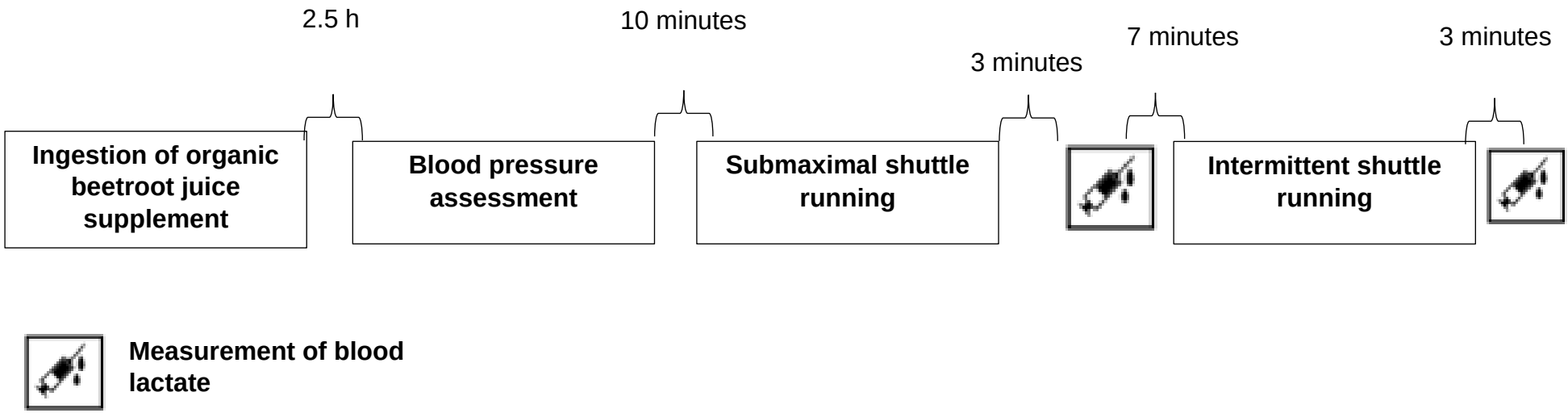


Figure 2 An illustration of the testing procedures during each experimental trial

Statistical analyses

A Shapiro-Wilk test was used to assess if the data was normally distributed. Changes in blood pressure across the four experimental trials were assessed using a one-way analysis of variance (ANOVA). Where any main effects were found, a post hoc analysis was performed using a paired t-test along with a Bonferroni adjustment used to correct for pairwise comparisons. Changes in VO_2, HR and RPE throughout the sub-maximal shuttle running protocols and post-exercise blood lactate concentration were assessed using four separate two-way ANOVA tests (Shuttle distance [2] x Supplement [2]). Changes in time to exhaustion, VO_2, HR, RPE and post Blood lactate during the LIST were assessed using a two-way ANOVA (Shuttle Distance [2] x supplement [2]). Where any main effects or interaction effects were found, post-hoc analyses were conducted using separate paired t-tests along with the Bonferroni adjustment. The alpha level of significance was set at $p = 0.05$ for main analyses and was divided by the number of comparisons for the Bonferroni adjustment during any post hoc analyses. All tests were conducted using SPSS for windows version 22.0 (IBM statistics). Alongside null hypothesis testing, effect sizes were calculated for each dependent variable to give an indication of the size of any differences which may have occurred. Effect sizes of 0.2, 0.6 and 1.2 were considered as small, moderate and large effects, respectively (Hopkins, Marshall, Batterham & Hanin, 2009).

Chapter 3.0

Results

NO_3^- supplementation and blood pressure

Figure 3 shows blood pressure changes across trials. There was no supplementation effect for resting systolic (F = 38.893, *p* = 0.567) or diastolic (F = 0.003, *p* = 0.961) blood pressure.

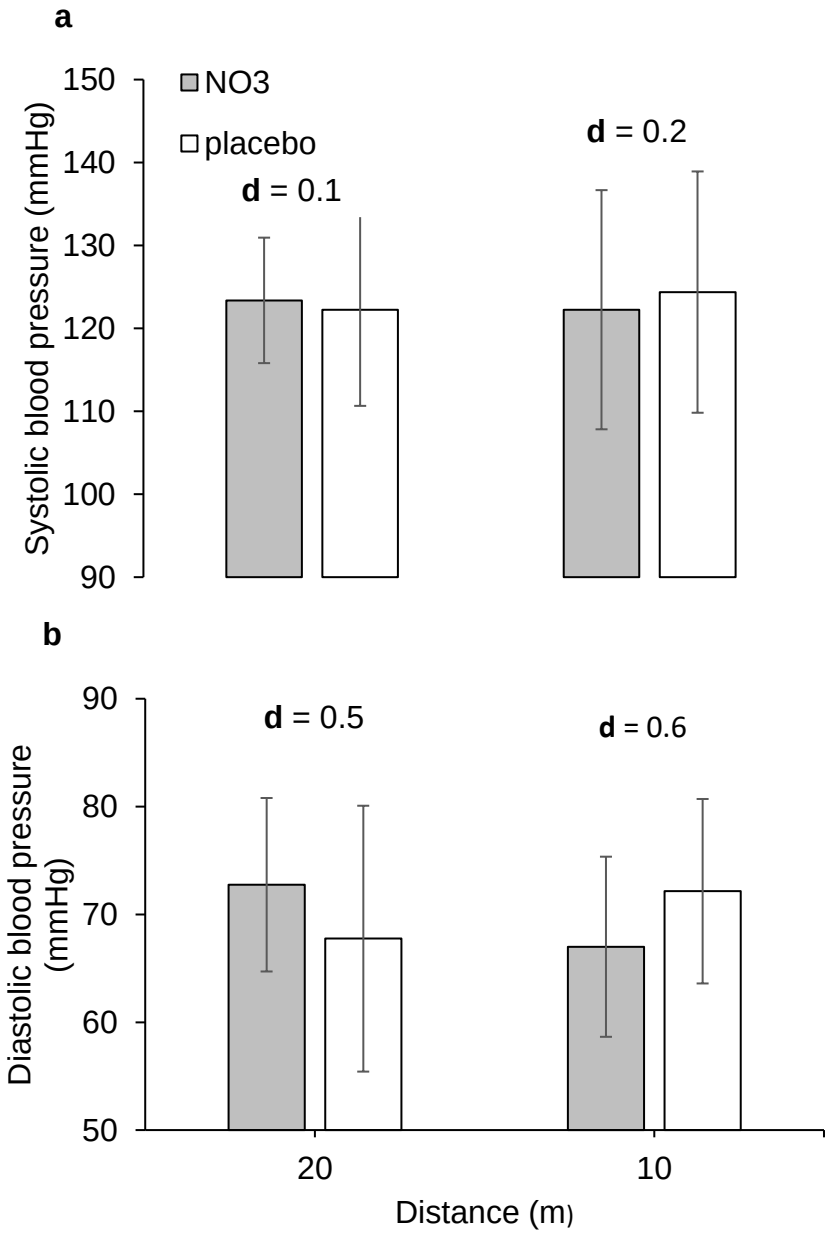
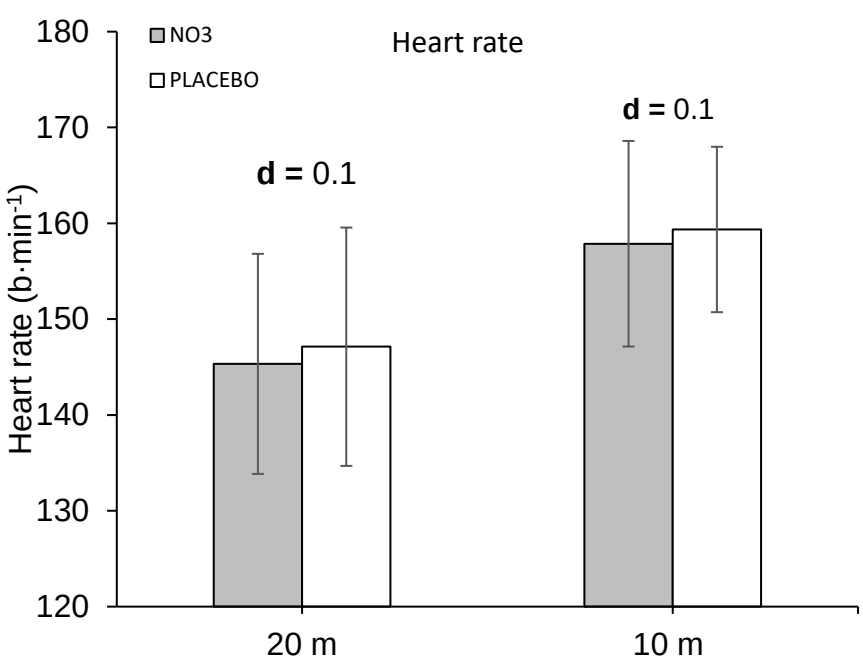
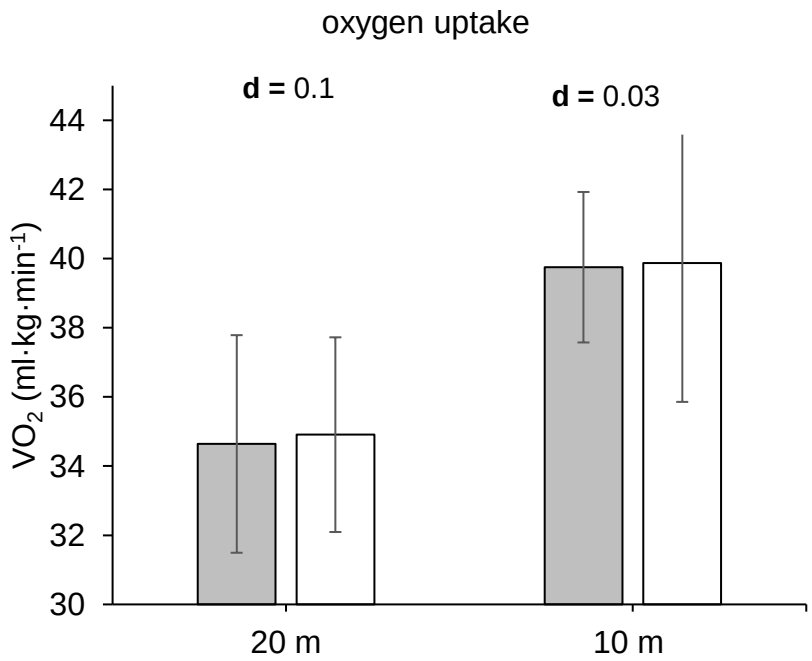


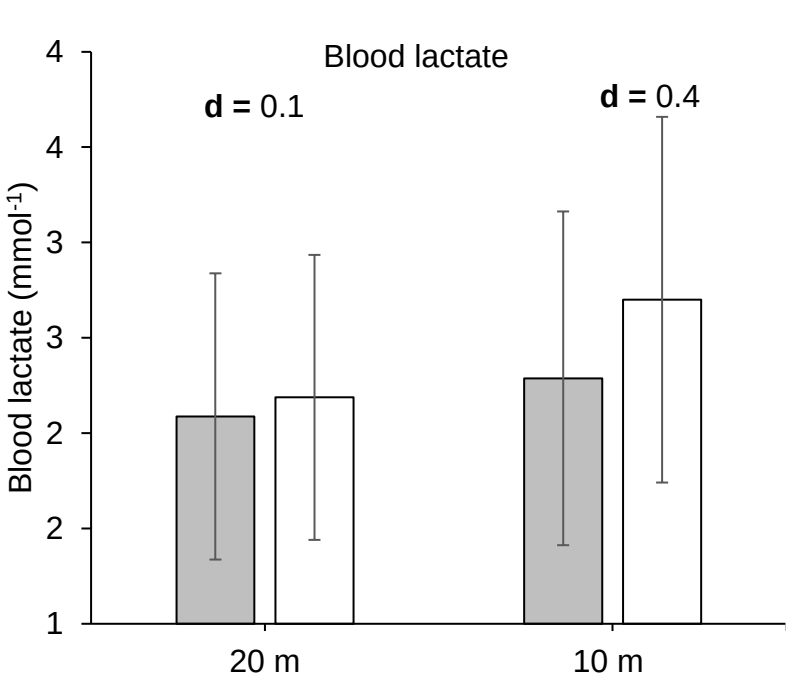
Figure 3 Changes in systolic (**a**) and diastolic (**b**) blood pressure following NO_3^- ingestion.

NO3- and the physiological and perceptual responses to submaximal shuttle running

Figure 4 shows the physiological and perceptual responses to submaximal shuttle running over 10 and 20 m following beetroot supplementation. There was a main effect for shuttle run distance on HR ($F = 12.324$, $p = 0.01$) and VO_2 ($F = 28.337$, $p = 0.0001$) during the submaximal shuttle runs, such that these variables were elevated during the 10 m trial. However, post-exercise blood lactate ($F = 4.492$, $p = 0.07$) and RPE ($F = 0.036$, $p = 0.01$) were unaffected by the shuttle run distance. NO_3^- supplementation did not affect HR ($F = 0.036$, $p =$), VO_2 ($F = 0.106$ $p = 0.754$), blood lactate ($F = 1.758$, $p = 0.27$) or RPE ($F = 2.778$, $p = 0.140$) and the effect of NO_3^- supplementation on these variables was not influenced by shuttle run distance (HR ($F = 0.03$, $p = 0.855$, VO_2 ($F = 0.10$, $p = 0.92$), blood lactate ($F = 0.436$, $p = 0.53$), RPE ($F = 4.667$, $p = 0.068$).







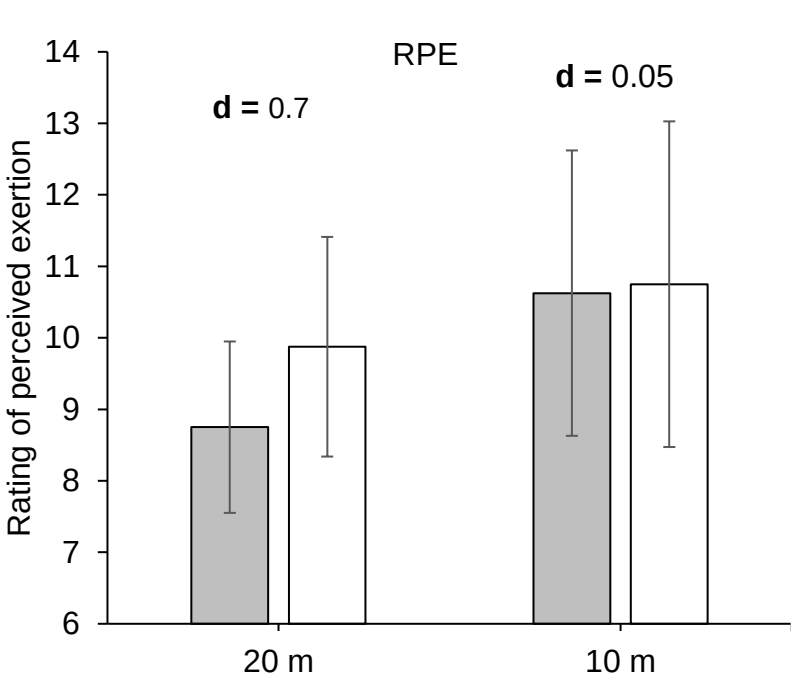


Figure 4 Changes in the **a)** heart rate**, b)** oxygen uptake, **c)** blood lactate and **d)** RPE response to submaximal shuttle running after NO_3^- ingestion.

d = effect size of the change, where an effect of 0.2, 0.6 and 1.2 are classified as a small, moderate and large effect, respectively.

The effects of NO_3^- ingestion on intermittent shuttle running

Changes in time to exhaustion over the intermittent shuttle running are shown in figure 5, whilst the physiological and perceptual responses to intermittent shuttle running are displayed in table 1. There was a main effect for shuttle run distance on time to exhaustion ($F = 67.101$, $p = 0.000$), VO_2 ($F = 75.836$, $p = 0.000$), end RPE ($F = 8.750$, $p = 0.025$) and average RPE ($F = 6.427$, $p = 0.03$), such that these variables were lower during the 10 m trial. Blood lactate ($F = 5.037$, $p = 0.06$) and HR ($F = 4.257$, $p = 0.07$) were unaffected by shuttle run distance. NO_3^- did not affect time to exhaustion ($F = 3.075$, $p = 0.12$), HR ($F = 0.758$, $p = 0.41$), VO_2 ($F = 0.452$, $p = 0.523$), blood lactate ($F = 0.407$, $p = 0.57$), end RPE ($F = 0.636$, $p = 0.45$) or average RPE ($F = 0.636$, $p = 0.451$) during the 10 m or 20 m shuttle run trials and the effect of NO_3^- on these variables was not influenced by the distance of the shuttle runs (time to exhaustion ($F = 2.128$, $p = 0.18$), heart rate ($F = 0.38$, $p = 0.55$), VO_2 ($F = 1.017$, $p = 0.347$), blood lactate ($F = 0.000$, $p = 0.989$), end RPE ($F = 0.636\ 7$, $p = 0.451$) or average RPE ($F = 0.636$, $p = 0.45$).

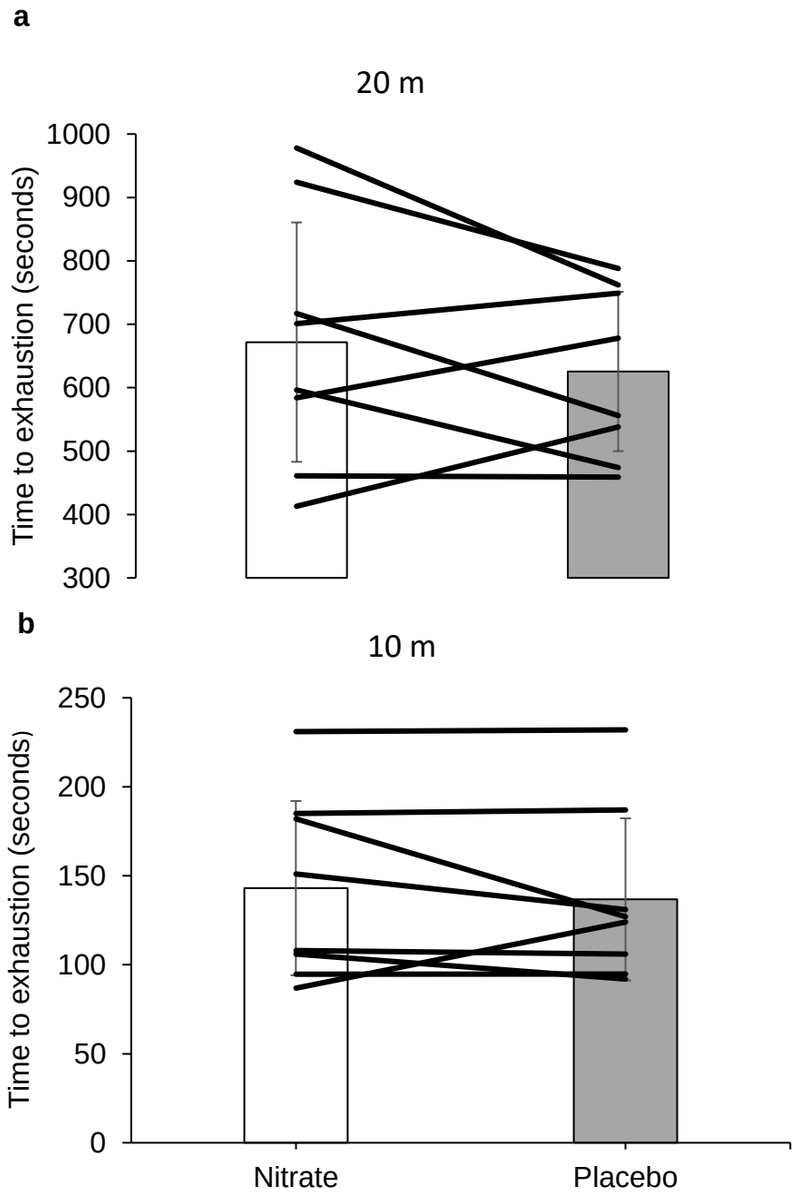


Figure 5 Changes in time to exhaustion during 20 m (**a**) and 10 m (**b**) intermittent shuttle running following NO_3^- ingestion.

Table 1 Changes in the physiological responses to intermittent shuttle running following NO_3^- ingestion

	10 m			20 m		
	Nitrate	**Placebo**	**d**	**Nitrate**	**Placebo**	**d**
TTE	143.07 ± 48.95	136.73 ± 45.55	0.1	671.8 ± 188.77	625.51 ± 125.61	0.6
Heart rate	149.84 ± 52.49	165.19 ± 9.37	0.4	176.11 ± 3.01	178.61 ± 5.91	0.5
VO_2	31.74 ± 4.78	29.34 5.11	0.4	44.5 ± 4.09	44.81 ± 3.59	0.1
RER	0.93 ± 0.06	0.93 ± 0.06	0.1	0.97 ± 0.03	0.96 ± 0.004	0.1
Blood lactate	13.08 ± 2.91	12.64 ± 2.86	0.1	9.61 ± 2.46	9.19 ± 2.51	0.2
Average RPE	16.57 ± 1.65	16.90 1.49	0.2	15.65 ± 0.76	15.43 ± 1.02	0.2
End RPE	18.88 ± 1.27	18.63 ± 1.11	0.2	20	20	0.2

Chapter 4.0

Discussion

The current study investigated the effect of NO_3^- supplementation on the physiological responses to submaximal shuttle running and on performance during intermittent shuttle running involving different numbers of directional changes. The main findings of the current study were: 1) Increasing the number of directional changes during the shuttle running led to an elevated metabolic and physiological demand, as indicated by an elevated VO_2 and HR response and a 125% decrease in time to exhaustion during the 10 m protocol and 2) NO_3^- had no effect on blood pressure and the physiological and perceptual responses to submaximal shuttle running and performance during intermittent shuttle running.

Previous studies have demonstrated that NO_3^- supplementation increases plasma NO_2^- levels and lowers blood pressure in healthy humans by increasing NO production and improving blood flow and vasodilation (Vanhatolo et al., 2010; Larsen et al., 2007; Webb et al., 2008). In the current study, the effects of NO_3^- supplementation on blood pressure were unclear, with no changes in systolic diastolic blood pressure occurring in response to NO_3^- supplementation, which was in contrast to our hypothesis. One explanation for the absence of a change in blood pressure may be that the NO_3^- dose and length of supplementation was insufficient to increase plasma NO_2^- levels and subsequently NO production to induce changes in blood flow. Indeed, this explanation is consistent with the lack of observed change in the physiological responses and performance during shuttle running that will be discussed herein.

VO_2 during the 10 m shuttle run trial was elevated by 14% in comparison to the 20 m version, and this was accompanied by an 8.2% increase in HR. This finding is consistent with previous studies which have reported that increasing the number of directional changes during shuttle running elevates the metabolic and physiological demand (Buchheit et al., 2011; Dellal et al., 2010; Stevens et al., 2015). As the number of directional changes increased, participants would have been required to spend more time decelerating and reaccelerating to achieve the same average shuttle run speed (Ashton & Twist, 2015). These increased number of acceleration phases would increase the energetic demand (Di Prampero et al., 2012) and increase the recruitment of less efficient higher order motor units (Ashton & Twist, 2015), leading to a higher metabolic response. However, it's interesting to note that we failed to observe an increase in blood lactate in response to the 10 m trial, which is in contrast to previous studies which reported an elevated blood lactate response to shuttle running involving more directional changes (Dellal et al., 2010; Ashton & Twist, 2015), and which would have been expected if an increased recruitment of type II motor units had occurred (Golinick, Warwrick & Hodgson, 1986; Ashton & Twist, 2015). However, whereas previous studies reported increased blood lactate values in response to an increased number of directional changes during high intensity intermittent exercise protocols (Ashton & Twist, 2015; Dellal et al., 2010; Buchheit et al., 2010), the current study used submaximal shuttle running at 60% of participant's predicted $VO_{2peak.}$ Thus, in line with the size principle (Enoka & Stuart, 1984), it's possible that the lower exercise intensity and running speed employed here allowed the increased demand of changing direction to be met predominantly by an increased recruitment of type I fibres and an increase in oxidative metabolism.

Despite the elevated oxygen uptake response, in contrast to our hypothesis, VO_2 remained unchanged following NO_3^- ingestion during both the 10 and 20 m protocol. This finding is in contrast to previous studies which reported a decrease in oxygen uptake during submaximal exercise (Larsen et al., 2007; Bailey et al., 2009; Vanhatolo et al., 2010), but is in line with previous studies which reported no change in oxidative efficiency following NO_3^- ingestion (Aucouturier, Boissiere, Chaouch, Cuvellier & Gamelin, 2015; Wilkerson et al., 2012; Peacock et al., 2012). One explanation why NO_3^- may not have improved the oxygen uptake response to exercise is that the acute dosing regime used may not have been sufficient to induce mitochondrial changes (Jones, 2014). Although a decrease in oxygen uptake in response to acute NO_3^- ingestion has been observed previously (Vanhatolo et al., 2010), changes in mitochondrial protein status and a decrease in the PCr cost of exercise that are indicative of a shift towards improved oxidative metabolism have only been reported following chronic doses of NO_3^- (Bailey et al., 2009; Larsen et al., 2011), and it has been suggested that in some participants a chronic dosing regime may be required to induce changes in mitochondrial efficiency (Jones, 2014). Secondly, the reduction of NO_2^- to NO is facilitated during intense exercise during conditions of low muscle Ph and low PO_2 tension (Lundberg et al., 2008; Moncada & Higgs, 1993). Thus, it's possible that, as indicated by the absence of an increase in blood lactate and an increase in oxygen uptake during the 10 m trial, the improved muscle oxygenation and the lack of metabolic acidosis failed to optimise the reduction of NO_2^- to NO. Future research should investigate the effect of NO_3^- supplementation on the metabolic responses to shuttle running following longer dosing regimes and at higher exercise intensities.

Previous studies have demonstrated that shuttle running involving more directional changes elicits a greater perceptual response (Ashton & Twist, 2015; Dellal et al., 2011). In the current study, although not reaching statistical significance, there was a trend for RPE to be 16% higher during the 10 m trial in comparison to the 20 m trial, suggesting that participants were perceiving exercise to be more difficult with more directional changes. Effort perception during exercise has been argued to be the result of afferent feedback signals sent from various physiological systems, including the heart, the lungs and the muscle, which are integrated within control centres in the brain to regulate effort (Gibson et al., 2006). Thus, it's possible that the trend for a greater RPE response was the result of the increased metabolic and physiological demand of the 10 m shuttle run protocol. Secondly, the added task complexity associated with changing direction may have increased participants' perceptions of mental fatigue (Ashton & Twist, 2015; Marcora, 2009). Following NO_3^- ingestion, RPE remained unchanged, suggesting that NO_3^- failed to attenuate the perceptual demand of shuttle running. Comparison with previous studies is difficult, as few studies have reported changes in RPE during submaximal exercise following NO_3^- ingestion (Larsen et al., 2007; Bailey et al., 2009). However, it's possible that the failure of NO_3^- to attenuate the perceptual response was related to the lack of effect of NO_3^- on the physiological responses to exercise.

It was hypothesised that NO_3^- supplementation would improve performance and that NO_3^- would be more ergogenic during the 10 m trial based on the theory that NO_3^-'s ergogenicity is specific to type II fibres (Hernandez et al., 2012; Jones et al., 2016). However, there were no changes in running time to exhaustion following NO_3^- supplementation, indicating that NO_3^- did not improve performance. This finding is in contrast to previous studies which have reported that NO_3^- supplementation

improves work performed during intermittent shuttle running protocols (Wylie et al., 2013; Thompson et al., 2016; Nyakayiru et al., 2017), but is line with studies which reported no changes in intermittent or continuous exercise performance following NO_3^- ingestion (Martin et al., 2014; Cermak et al., 2012; Wilkerson et al., 2012; Peacock et al., 2012). Although not reaching statistical significance, there was a 7.1% and 4.9% increase in time to exhaustion during the 20 m and 10 m trials, respectively, and performance improved for four participants across both trials, whilst remaining unchanged or becoming impaired for the remaining participants. However, given that it wasn't the same participants who improved or deteriorated performance across both the 10 m and 20 m shuttle run trials, it is unlikely that these participants can be classified as responders/non-responders to NO_3^- supplementation, but that the results were the consequence of a large within-subject variation (Atkinson & Batterham, 2015). Notwithstanding, factors which may explain why some participants may have responded to NO_3^- supplementation whilst others didn't include participant's training status, with NO_3^- displaying greater effectiveness amongst those less well-trained (Porcelli et al., 2015); an increased number of type II muscle fibres and lower muscle capiliarisation (Jones, 2014; Dominguez et al., 2018) and the adoption of a diet lower in NO_3, resulting in lower baseline plasma NO_2^- levels and an increased capacity to increase NO_2^- levels (Poveda, 1997). Future research should investigate whether true individual responders to NO_3^- supplementation exist by performing repeated trials across participants, and should investigate mechanisms which may explain any individual response to NO_3^- supplementation. Factors which may explain any improvements observed amongst participants in the current study include an increase in blood flow to type II motor units and improved phosphocreatine replenishment (Ferguson et al., 2014; Wylie et al., 2013), improved

intracellular potassium and calcium handling and an improved contractility of type II fibres (Hernandez et al., 2012; Wylie et al., 2013), and an increase in blood glucose uptake (Wylie et al., 2013).

Limitations

The findings of the current study are limited by the small sample size, which resulted in the study possessing a small amount of statistical power. Based on the effect size of 0.28 observed for the effect of NO_{3-} on intermittent shuttle run performance and the achieved sample size of 8, a post hoc power analysis revealed that the study achieved a statistical power level of 0.37. Indeed, in order to achieve the desired power level of 0.8, a total sample size of 19 participants would have been required. Thus, with a larger sample size and less within subject variation, it's possible that the study would have achieved a greater statistical power level, resulting in a lower likelihood of making a type 2 error (Cohen, 1992).

Another limitation is that whereas previous studies have assessed oxygen uptake following NO_{3-} ingestion at exercise intensities of each participant's individual gas exchange threshold (Breese et al., 2013; Bailey et al., 2009), the current study used the running speed equating to 60% of participants' predicted $VO_{2max.,}$ which was estimated to be in the moderate intensity exercise domain. However, it is unlikely that this exercise intensity resulted in each participant exercising in the moderate intensity domain, resulting in different physiological responses to the shuttle run protocols and NO_{3-} ingestion (Meyer, Gabriel & Kindermann, 1999; Mann, Lamberts & Lambert, 2013).

Conclusion

The current study assessed the effect of NO_3^- supplementation on the submaximal responses to shuttle running and on performance during intermittent shuttle running involving different numbers of directional changes. The current study adds to the current body of literature which shows that increasing the number of directional changes during shuttle running increases the physiological demand. Such knowledge should be taken into consideration by sport scientists and coaches when planning training programmes and monitoring training load. Despite the increased metabolic demand, NO_3^- did not impact upon the physiological responses to submaximal shuttle running or performance during intermittent shuttle running. These findings suggest that NO_3^- supplementation may not be effective in attenuating the increased physiological demand of directional changes and improving team sports performance.

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Appendices

Appendix 1: Ethical approval

Approval 2017/18

University of Chester

Faculty of Medicine, Dentistry and Life Sciences
Research Ethics Committee

frec@chester.ac.uk

Thursday, 21 June 2018

Ben Francis
14 Leonard Street
Chester
Cheshire
CH1 4BW

Dear Ben,

Study title: The effect of dietary nitrate supplementation on intermittent shuttle running performance involving different numbers of directional changes.
FREC reference: 1401/18/BF/SES
Version number: 1

Thank you for sending your application to the Faculty of Medicine, Dentistry and Life Sciences Research Ethics Committee for review.

I am pleased to confirm ethical approval for the above research, provided that you comply with the conditions set out in the attached document, and adhere to the processes described in your application form and supporting documentation.

The final list of documents reviewed and approved by the Committee is as follows:

Document	Version	Date
Application Form	1	March 2018
Appendix 1 – List of References	1	March 2018
Appendix 2 – Summary CV for Lead Researcher	1	March 2018
Appendix 3 – Risk Assessment	1	March 2018
Appendix 4 – Participant Information Sheet [PIS]	4	June 2018
Appendix 5 – Participant consent form(s)	1	March 2018
Appendix 6 – Copies of advertisement material(s)	1	March 2018
Appendix 7 – Health screening document	1	March 2018
Appendix 8 – Food Diary	1	March 2018
Appendix 9 – List of nitrate rich foods for participants to avoid	1	March 2018
Appendix 10 – G*Power software showing required sample size	1	March 2018
Appendix 11 – Schematic of study design	1	March 2018

Appendix 2: Participant information sheet

Participant information sheet

University of Chester

The effects of nitrate supplementation on intermittent shuttle running performance involving different numbers of directional changes

You are being invited to take part in a research study. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Thank-you for reading this.

What is the purpose of the study?

The project is designed to assess if beetroot juice can improve exercise performance in team sports and if it can improve performance during sports which involve more directional changes.

Why have I been chosen?

You have been chosen because you are a team sport athlete, aged between 18-25. who regularly trains and competes as part of your sport.

Do I have to take part?

It is up to you to decide whether or not to take part. If you decide to take part, you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part, you are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect you in any way.

What will happen to me if I choose to take part?

If you choose to take part in this study, you will visit for testing on 5 separate occasions, with approximately 5 days apart. On the first visit, you will be asked to provide consent and to complete a questionnaire and the researcher will assess your resting blood pressure and heart rate to ensure that it is safe for you to take part in the research. You will also have your body mass and height assessed. Following this, you will perform the bleep test, which will involve you running in between two cones at a progressively increasing speed regulated by the noise of an audio tape, until you are no longer able to keep up the speed.

On the following four visits of the study, before arriving for testing, you will receive 70 ml of beetroot juice to ingest 2.5 hours before testing. On two of these occasions, the beetroot juice will contain a substance called nitrates and on the other two occasions, the beetroot juice will contain no nitrates. You will not be permitted to know which supplement does and does not contain nitrates until after the study, and both types of beetroot juice will taste exactly the same. There are no long-lasting side effects of ingesting beetroot juice or nitrates and nitrates are found in high amounts in green leafy vegetables and whole beetroot. However, you may observe that your urine is a pink/ red colour. This is nothing to worry about, and this will pass 1-2 days later.

Two and a half hours after ingesting the beetroot juice, you will perform measurements of resting blood pressure followed by two shuttle running protocols. The first shuttle running test will involve you running for five minutes at two relatively easy and submaximal speeds. Ten minutes after the first shuttle running, you will start the second shuttle running test. On this test, the running speed fluctuates between higher and lower running speeds. You will be asked to run until you become voluntarily fatigued to maintain the speed required by the test. On two of these visits, the cones which you are running between will be 20 m apart; whilst on the other two visits, they will be 10 m apart and you will be required to perform more changes of direction. Throughout all running tests, you will wear a heart rate monitor and a gas analysis mask and after the test, a small amount of blood will be taken from your fingertip after each running protocol by the researcher providing a small prick to your finger, which will induce a slight sharp pain that will dissipate almost immediately. You will be asked to provide consent for this to happen on the consent form. In between visits, you will be asked to refrain from performing strenuous exercise and you will be asked to refrain from foods such as beetroot and green leafy vegetables and caffeine-containing foods, and you will be asked to complete a food diary, documenting your dietary intake, in order to monitor this.

What are the possible disadvantages and risks of taking part?

The time commitment for this study is relatively high and as you will be asked to refrain from certain foods and from performing strenuous exercise in between visits. Thus, there will be some disturbance to your everyday life/routine and your training practice as part of your sport. Furthermore, as a result of performing strenuous exercise, you will experience some physical discomfort during exercise, such as breathlessness, exhaustion, exercise-induced sickness and dizziness. You may also experience some muscle fatigue and soreness in the days after the protocol. However, all of these physical symptoms are normal experiences during exercise and are likely what you experience during training or matches and in the days after. There is some risk that you may become injured during the protocol, however this risk is the same as that which you would experience taking part in your sport.

What are the possible benefits of taking part?

You will be taking part in research which aims to understand how to improve your nutritional intake to improve performance in your sport. You will also receive data and information regarding your current fitness levels, which you may use to improve your training and performance.

What if something goes wrong?

If you wish to complain or have any concerns about any aspect of the way you have been approached or treated during the course of this study, please contact the Dean of the Faculty of Medicine, Dentistry and Life Sciences, at the address of: The University of Chester Parkgate Road campus, Chester, CH1 4BJ; or contact on the telephone number of 01244 513208.

Will my taking part in the study be kept confidential?

All information which is collected about you during the course of the research will be kept strictly confidential so that only the researcher carrying out the research will have access to such information.

Participants should note that data collected from this project may be retained and published in an anonymised form. By agreeing to participate in this project, you are consenting to the retention and publication of data.

What will happen to the results of the research study?

The results will be written up as part of a masters research project and may be submitted for publication in the form of a scientific journal article. You will not be named in any report and you will be asked to provide written consent to ensure that you are happy with the publication of your data in anonymous form.

Who is organising the research?

The research is being conducted as part of a masters' dissertation project in Sports Physiology within the Department of Sport and Exercise Sciences at the University of Chester, which exists within the Faculty of Dentistry, Medicine and Life sciences at The University of Chester. The study is organised with supervision from the department, by Ben Francis, a MSc student.

Who may I contact for further information?

If you would like more information about the research before you decide whether or not you would be willing to take part, please contact:

Ben Francis: 1408861@Chester.ac.uk

Thank you for your interest in this research.

Appendix 3: Participant informed consent sheet

Title of Project: The effects of dietary nitrate supplementation on intermittent shuttle running performance involving different numbers of directional changes

University of Chester

Name of Researcher: Ben Francis

Please initial box

1. I confirm that I have read and understand the information sheet for the above study and have had the opportunity to ask questions. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason and without my legal rights being affected. ☐

3. I agree to take part in the above study. ☐

_____	_____	_____
Name of Participant	Date	Signature
_____	_____	_____
Researcher	Date	Signature

1 for participant; 1 for researcher

Appendix 4: List of nitrate-rich foods participants were asked to abstain from

List of nitrate-rich foods

Throughout taking part in this study, please avoid consuming any of the following foods which are classed as high or very high in nitrates

Nitrate	Content (per kg fresh vegetable)	Common Vegetables
Very High	2500 mg/40 mmol	Beetroot and beetroot juice, celery, lettuce, rocket, spinach
High	1000-2500 mg/18-40 mmol	Chinese cabbage, celeriac, endive, leek, parsley, kohlrabi,
Moderate	500-1000 mg/9-18 mmol	Cabbage, dill, turnips, carrot juice
Low	200-500 mg/3-9 mmol	Broccoli, carrot, cauliflower, cucumber, pumpkin, V8 vegetable juice,
Very low	<200 mg/< 3 mmol	Asparagus, artichoke, broad beans, green beans, peas, capsicum, tomato, watermelon, tomato, sweet potato, potato, garlic, onion, eggplants, mushroom